<Preliminary>Endo-1,4-β-glucanase in Suspension-cultured Poplar Cells

AUTHOR(S):

OHMIYA, Yasunori; NAKAMURA, Shingo; SAKAI, Fukumi; HAYASHI, Takahisa

CITATION:

OHMIYA, Yasunori ...[et al]. <Preliminary>Endo-1,4-β-glucanase in Suspension-cultured Poplar Cells. Wood research : bulletin of the Wood Research Institute Kyoto University 1994, 81: 5-7

ISSUE DATE:

1994-09-30

URL:

http://hdl.handle.net/2433/53251

Preliminary

Endo-1, 4-β-glucanase in Suspension-cultured Poplar Cells*[1]

Yasunori OHMIYA*[2], Shingo NAKAMURA*[2],
Fukumi SAKAI*[2] and Takahisa HAYASHI*[2]

(Received June 1, 1994)

Keywords: Endo-1, 4-β-glucanase, *Populus alba*, Wall-bound enzymes, N-Termini sequence.

Introduction

Plant endo-1, 4-β-glucanase (EC3.2.1.4) activities are closely related to various physiological aspects of plant growth, *i.e.* cell-wall loosening in the primary wall, vascular differentiation, leaf abscission, fruit ripening and symbiosis. The endo-1, 4-β-glucanases may play a part in hydrolyzing wall materials during these processes.

Suspension-cultured poplar cells generate and secrete endo-1, 4-β-glucanase activities which have been detected in the cell wall and extracellular culture medium. When auxin-starved cells were cultured in a medium containing 2, 4-D, the activity of the β-glucanase bound to cell walls increased and was maintained during growth, whereas the activity of the enzyme secreted into the extracellular culture medium increased markedly up to the mid-log growth phase and then disappeared in the stationary growth[1)]. The extracellular β-glucanase was purified from poplar culture medium[2)] and its cDNA was isolated[3)]. In this paper, we have isolated the wall-bound endo-1, 4-β-glucanase from suspension-cultured poplar cells for the first time.

Results and Discussion

Poplar cells were homogenized and extracted five times with 20 mM sodium phosphate buffer (pH 6.2). The activity was assayed viscometrically at 35°C with 0.1 ml of enzyme preparation plus 0.9 ml of 20 mM sodium phosphate buffer (pH 6.2) containing 0.6% (w/v) carboxymethylcellulose. The specific activity of enzyme preparation extracted with the buffer containing 0.5 M NaCl was increased to 3.26×10^3 unit mg^{-1} protein compared with other fractions after extraction with the buffer containing 0.1 M NaCl of wall preparations.

*[1] This report was presented at the Japanese Society of Plant Physiologists, Tukuba, Japan, Mar. 28–30, 1994.

*[2] Laboratory of Gene Expression. Wood Research Institute, Kyoto University, Uji, Kyoto, 611 Japan.

Ninety three per cent of total activity was recovered in the precipitate between 40% and 60% saturated ammonium sulfate. The specific activity was 3.18×10^{3} unit mg^{-1} protein at this stage.

Wall-bound endo-1, 4-β-glucanase was purified to homogeneity on SDS-PAGE by cation exchange, hydrophobic, and gel filtraion chromatography. Although only a single peak of whole proteins (52 kDa) with endo-1, 4-β-glucanase activity was obtained at pH 6.2 from the gel filtraion, several proteins were separated by the same gel filtraion by changing the pH to 7.5, one of which contained β-glucanase activity corresponding to a certain molecular size of 48 kDa. The final preparation of endo-1, 4-β-glucanase appeared to be homogeneous both on SDS-PAGE (47 kDa) and isoelectric focusing (pI 5.6).

The enzyme decreased very rapidly the specific viscosity of carboxymethylcellulose solutions at the early stage of the reaction, whereas the ruducing power of the incubation mixture increased at a low but linear and constant rate, indicating typical endo-hydrolysis. The K_m and V_{max} values with carboxymethylcellulose were 1.2 $mg\cdot ml^{-1}$ and 280 units, respectively. The purified enzyme was active between pH 6 and 7, with an optimum pH of 6.5 and stable at least for one month at pH 6–7.5 with 10 mM phosphate buffer at 4°C. Under the standard conditions, the enzyme did not change the reducing power of Avicel, pustulan, laminarin and cellobiose. The enzyme specifically cleaved the 1, 4-β-glycosyl linkages of caboxymethylcellulose, phospho-swollen cellulose, lichenan, xyloglucan and xylan, but the latter two substrates were hydrolyzed very slowly.

The N-terminal sequence of the purified enzyme was determined to 30 residues as follows;

FTSQDYADALEISILFFEGQRSGKLPLNQR...

Examining the deduced amino acid sequences from the cDNA of avocado fruit ripening and bean abscission endo-1, 4-β-glucanases, sequences in its translated regions with homology at 73% and 70%, respectively, were found to the N-terminal sequence of the enzyme. The fact that the N-terminal sequence of the enzyme exhibits homology only to a specific internal sequence of the deduced amino acid sequences of avocado fruit ripening and bean abscission endo-1, 4-β-glucanase cDNAs indicate that the enzyme with a molecular size of 47 kDa may be a proteolytic fragment of a larger gene product.

The physical and enzymatic properties of the enzyme was distinguishable from extracellular poplar endo-1, 4-β-glucanase in spite of their isoelectric, kinetic similarity. Extracellular enzyme was purified by using anion-exchange chromatographies, and there is a difference in the molecular size between gel filtration (40 kDa) and SDS-PAGE (50 kDa). Wall-bound endo-1, 4-β-glucanase has slightly higher activities against lichenan, xyloglucan and xylan than extracellular endo-1, 4-β-glucanase. The N-termini between wall-bound and extracellular β-glucanases showed 90% homologies. These findings suggest that wall-

bound endo-1, 4-β-glucanase is the isozyme of extracellular endo-1, 4-β-glucanase in suspension-cultured poplar cells.

An avocado fruit ripening endo-1, 4-β-glucanase has been shown to belong to a small gene family by genomic blot analysis. This suggests the existence of endo-1, 4-β-glucanase subfamilies expressed in different tissues, different stages of development, and slightly different functional properties. Multiplicity of enzymes have also been reported in many plants, and when two proteins exhibit highly similar but not fully identical amino acid sequences, these are probably due to distinct genes. These findings suggest that wall-bound and extracellular endo-1, 4-β-glucanases are members of multigene family caused by two distinct genes and they probably show varieties of organ-specific expression patterns.

Acknowledgements

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

References

1) S. NAKAMURA and T. HAYASHI: *Mokuzai Gakkaishi,* **39**, 1056–1061 (1993a).
2) S. NAKAMURA and T. HAYASHI: *Plant Cell Physiol.,* **34**(7), 1009–1013 (1993b).
3) S. NAKAMURA, H. MORI, F. SAKAI and T. HAYASHI: *ibid.,* **35** (Supplement), s33 (1994).